Plumage coloration and personality in early life: sexual differences in signalling

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Several studies have shown that melanin-based traits play a crucial role in social contexts as they are associated with dominance, personality and social behaviour. However, most of these studies have focused on adults, and the role of these traits in juveniles has rarely been explored. Here, we explore the association between two melanin-based traits and nestling personality in Common Kestrels Falco tinnunculus. Our results show that female nestlings with blacker plumages displayed bolder personality traits, providing evidence of sex-dependent phenotypic integration of these two traits in males and females. We consider that this differential integration may arise from different selection pressures acting on males and females on plumage coloration during adulthood and that nestling coloration can act as a status signal within the juvenile age-class.

Keywords: coloration, Common Kestrel, dominance, Falco tinnunculus, melanin-based traits, personality, phenotypic integration.

Coloured traits are one of the most conspicuous mechanisms used by animals to convey information about their quality or behaviours (Hill & McGraw 2006). There are a multitude of studies showing that the expression of different coloured traits can be associated with individual quality in different contexts (Hill 1991). However, nearly all of the studies exploring these issues have focused on adult individuals, and the role of juvenile coloration remains mostly unexplored (Moreno & Soler 2011). In many species, juvenile plumages are strikingly different from those exhibited by adults. In addition to the function of crypsis, their main role has usually been associated with signalling subordination to reduce aggressiveness from adults (Lyon & Montgomerie 1986). However, the function of juvenile coloration within a communication context is unclear and some recent studies have shown that juvenile colorations can also work as badges of status, signalling dominance within the juvenile age-class (Jones 1990, Vergara & Fargallo 2008, Tringali & Bowman 2012, Fargallo et al. 2014). Alternatively, it has also been stated that juvenile coloration can work in a parent–offspring communication context, signalling individual quality to modulate the allocation of parental resources (Penteriani et al. 2007, Galván et al. 2008). Both perspectives suggest that juvenile coloration can be a channel to communicate different aspects of the bearers within and between age-classes, such as quality or dominance. Nonetheless, further research is needed to understand the adaptive function of juvenile coloration under social pressures.

Melanin is the most common pigment in vertebrates, and melanin-based traits have an important signalling role in social contexts (Senar 2006, Roulin 2016). Melanin-based signals are particularly relevant in signalling status, providing information about the competitive capabilities of the bearers (Rohwer 1975, Senar et al. 1993). Several studies have, for example, shown that an enhanced expression of melanin-based traits leads to the discrimination of dominant and subordinate individuals (Senar et al. 1993, López-Idiáquez et al. 2016a). It has been suggested that this association is rooted in the combined effects of testosterone, which modulates both melanogenesis (Wilson 1983, Fargallo et al. 2007b) and behaviour (Collis & Borgia 1992, Alonso-Alvarez & Velando 2001), or by pleiotropy (Ducrest et al. 2008). Little is known, however, about the colour–behaviour relationship in early life (Vergara & Fargallo 2008, Fargallo et al. 2014, Dreiss et al. 2016, Roulin et al. 2016).

Coloration, especially melanin-based coloration, has been found to covary with other phenotypic traits, including behaviour (Ducrest et al. 2008, McKinnon & Pierotti 2010). This phenotypic integration has been explained through several mechanisms, e.g. functional, developmental and genetic interactions, including the combined effects of hormones and pleiotropy in the melanocortin system (Pigliucci & Preston 2004, Ducrest et al. 2008, Ketterson et al. 2009, McKinnon & Pierotti 2010). Personality can be described as the individual differences in the response to certain stimuli across time.

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and contexts (Gosling 2001). The evolutionary importance of personality has been stressed by its association with fitness (Réale et al. 2000), suggesting that it can be modulated by natural selection (Smith & Blumstein 2007). Nevertheless, studies exploring the evolutionary consequences of personality have focused on adult individuals, and in spite of the fitness consequences of behaviour during the first stages of life (Le Galliard et al. 2013, Rödel et al. 2014), very little is known about the factors that drive variation in personality in early life.

Several studies have reported that personality usually covaries with the expression of coloration, suggesting that these two traits can be phenotypically integrated (van den Brink et al. 2012a, Da Silva et al. 2013, Fargallo et al. 2014, Velando et al. 2017), such that selection can favour correlated responses on multivariate phenotypes (Pigliucci & Preston 2004). However, there is variation among individuals within a population on the level of trait integration. For example, phenotypic integration may be different in males and females due to sex-specific selection on life-history profiles and underlying physiological systems (Fairbairn et al. 2007, Ketterson et al. 2009, Immonen et al. 2018). In sexually dimorphic species, male sexual ornaments are usually under strong directional selection, promoting reduced integration with other traits to obtain a stronger expression of the ornament (Pigliucci & Preston 2004). In addition, it is well documented that sexual ornaments show a greater sensitivity to environmental variation, making them more flexible or plastic and therefore less integrated (Pigliucci & Preston 2004). Importantly, in addition to sex-specific selection pressures, age-specific selection can also influence adult and juvenile phenotypes (Mank et al. 2010). Thus, sexual differences in behaviour, morphology or other traits (Badyaev 2002), and their integration, can be expected in the earlier stages of life (Fargallo et al. 2014, Velando et al. 2017). Hence, exploring the integration levels of coloration and behaviour during the first stages of life can identify the selective pressures influencing trait expression.

Here, we explored the association of personality (shy/bold axis) and two melanin-based traits, rump coloration and plumage blackness, in Common Kestrel Falco tinnunculus fledglings. This species is sexually dimorphic and females choose males exhibiting brighter plumages (Palokangas et al. 1994). In this species both rump coloration and plumage blackness are associated with different behavioural aspects – dominance (Vergara & Fargallo 2008) and anti-predator behaviour (van den Brink et al. 2012b), respectively – suggesting a link between coloration and personality (van den Brink et al. 2012b). We also studied a potential sex-dependent integration of personality and coloration which has been previously reported in other species (Fargallo et al. 2014). If coloration has a role in signalling status in chicks, we predicted a correlation between plumage coloration and boldness. Furthermore, as coloration works as an ornament in male Kestrels, we predicted plumage coloration would be less integrated with other phenotypic traits in males than in females.

METHODS

General procedures

The study was conducted in the Campo Azálvaro region, a treeless grassland located in central Spain, where 62 nestboxes are available for Kestrel breeding (Fargallo et al. 2001). Kestrels are medium-sized raptors, dimorphic in size and plumage coloration. Nestlings start developing their plumage when they are 2 weeks old and maintain it during the juvenile stage (approximately 1 year; Village 1990). During the breeding season, nests were visited to detect laying and hatching date, and the number of fledglings (mean = 4.15 ± 0.30, range = 1–6). When nestlings were 26 days old, we recorded their body mass (to the nearest g) and wing length (to the nearest mm), took a blood sample for molecular sexing and measured the proportion of grey coloration of the rump (Fargallo et al. 2007a). We also took a digital photograph to assess plumage blackness (see below). Fledging occurs on average at the age of 31 days in our population (Vergara & Fargallo 2008).

Blackness of nestling plumage

Photographs included the back and right wing of all nestlings and a scale to determine the size of the target traits and were analysed using Adobe PHOTOSHOP CS6. We measured four different traits: tail terminal bands (TTB), tail bars (TB), rump bars (RB) and wing blackness (WB; see Supporting Information, Figure S1). TTB corresponded to the mean value of the band width in the two central tail feathers. The values for TB and RB were calculated as the mean band width measured in three and four different feathers of the left side of the body, respectively. WB was measured as the proportion of black coloration in an area of the wing covers. All measurements were highly repeatable (r > 0.82; Appendix S1). We used a principal components analysis (PCA, following López-Rull et al. 2016) to obtain a proxy of plumage blackness.

Behavioural assays

When nestlings were 26 days old, we performed two behavioural tests (resulting in five different variables) to assess their personality following a standardized protocol (Appendix S2).

In the first test, we quantified the distance that nestlings covered in 1 min after leaving them on the ground.
just in front of the feet of the observer (escape distance). The nestling was then taken off the ground, and its response to being captured was recorded and scaled from 0 to 3 (capturing response). Score 0 was given to nestlings that did not respond, score 1 to those that tried to escape, score 2 to those that turned around and threatened with their talons, and score 3 to nestlings that turned around and attacked using their talons. We also performed a restraint test to evaluate nestling aggressive behaviour. Specifically, we offered and took away a gloved hand three times, as previously described in other species (Viñuela et al. 1995, Fargallo et al. 2014), counting the number of times the nestling attacked the gloved hand (number of attacks) and the intensity of the response (attack intensity) which we assigned to one of five categories: 0 corresponded to those individuals that did not attack; 1 to those that attempted to grab the gloved hand without touching it; 2 to those nestlings that grabbed and immediately released the gloved hand; 3 to those that grabbed the gloved hand but released it when the hand was taken away; and 4 to nestlings that grabbed strongly and did not release when the gloved hand was taken away. During the behavioural tests we determined nestlings’ resistance to handling using five levels (‘handling resistance’): 0 was assigned to offspring that remained calm and silent when handled; 1 when there was a small number of chirps and some writhing; 2 when there were a large number of chirps and some writhing during handling; 3 corresponded to nestlings that chirped and writhed during most of handling; and 4 was the maximum score, assigned to those nestlings that behaved restlessly, chirping and writhing, trying to peck the observer’s hand. The repeatability of the five variables was estimated by performing the same tests on the following day in a subset of 29 nestlings (Appendix S1). Our five behavioural traits were included in a PCA with the aim of obtaining a single axis (boldness) as a proxy of nestling personality.

**Statistical analyses**

We used linear models (LM) in R statistical software using the package lme4 (Bates et al. 2015). As a response variable, we included the first axis of the PCA obtained after including all behavioural traits. This variable was mean-centred for each year and nest (which we refer to as boldness,) to control for the variability associated within year and nest. As explanatory variables, we included plumage blackness, rump coloration, wing length, nestling sex and body mass, and the interactions between sex and the other explanatory variables. We calculated variance inflation factors (VIFs) using the package usdm (Babak 2015), without finding any evidence of collinearity (VIFs < 1.12, see Appendix S3). The combination of all variables was fitted and the more plausible models were selected using Akaike’s information criteria for small samples (AICc), using the package MuMln (Barton 2016). Models with smaller AICc values were preferred, and those models that differed in more than 2 units in relation to the smallest AICc were not considered further (Burnham & Anderson 2002).

**RESULTS**

**Principal components analyses**

First, the PCA of coloration retained a single axis (PC1 – plumage blackness) that explained 45.8% of the variance (Table 1). This axis was positively associated with all variables (Table 1); higher values of the axis represented blacker individuals. Secondly, the PCA of the behavioural traits retained only the first axis (PC1 – boldness), which explained 52.4% of the variance (Table 1). All behavioural traits included in ‘boldness’ were negatively correlated, except escape distance, which had a positive association (Table 1). This axis represents a shy/bold gradient, in which higher values are associated with shyer behaviours. Because this ‘negative’ association between the values of the axis and the behaviour may seem counterintuitive, we multiplied it by −1. In both cases we only retained the first axes, as the remaining axes had small eigenvalues (<1.03, Appendix S4) and were not easily biologically interpretable. However, as the second axes in each PCA had eigenvalues slightly larger than 1, we refitted all our models including those variables and found no significant association (see Appendix S5).

**Association between personality and coloration**

According to the AICc our results showed a sex-dependent association of boldness between plumage blackness and wing size (Table 2), although none of the best models included rump coloration. We further explored these models and split both interactions to explore the slope and directionality of the sex-dependent associations between boldness and plumage blackness or wing size. We found a statistically significant positive relationship between boldness and plumage blackness in females but not in males (Table 3, Fig. 1). We did not find any statistically significant relationship between boldness and wing size for either males or females (Table 3).

**DISCUSSION**

Our results show a positive association between boldness and plumage blackness in nestlings of Common Kestrels, suggesting that juvenile coloration may play a signalling role within a social context. The main function proposed for juvenile coloration is crypsis (Moreno & Soler 2011).
Variables (*g* terms were also included. Within a social context, it has been considered that the main function of juvenile plumages is to show subordination to reduce aggression from adults, or specifically that juvenile plumage has evolved as a solution to avoid adult male aggression (female mimicry hypothesis).

Table 1. Factor loadings of the (a) coloration and (b) behavioural variables included in the principal components analyses, and the percentage of variance explained by each component.

<table>
<thead>
<tr>
<th>Trait</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
<th>PC5</th>
<th>Explained variance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Coloration traits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terminal tail bands</td>
<td>0.242</td>
<td>0.866</td>
<td>0.435</td>
<td>0.034</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Tail bars</td>
<td>0.568</td>
<td>0.137</td>
<td>–0.629</td>
<td>0.511</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Rump bars</td>
<td>0.614</td>
<td>–0.092</td>
<td>0.097</td>
<td>–0.777</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Wing blackness</td>
<td>0.491</td>
<td>–0.471</td>
<td>0.636</td>
<td>0.363</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Explained variance (%)</td>
<td>45.84</td>
<td>25.53</td>
<td>16.50</td>
<td>12.13</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>(b) Behavioural traits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escape handling resistance</td>
<td>0.130</td>
<td>0.794</td>
<td>–0.592</td>
<td>0.008</td>
<td>–0.019</td>
<td></td>
</tr>
<tr>
<td>Capturing response</td>
<td>–0.417</td>
<td>–0.339</td>
<td>–0.555</td>
<td>–0.633</td>
<td>–0.012</td>
<td></td>
</tr>
<tr>
<td>Number of attacks</td>
<td>–0.520</td>
<td>0.345</td>
<td>0.366</td>
<td>–0.149</td>
<td>–0.672</td>
<td></td>
</tr>
<tr>
<td>Attack intensity</td>
<td>–0.552</td>
<td>0.301</td>
<td>0.258</td>
<td>–0.038</td>
<td>0.731</td>
<td></td>
</tr>
<tr>
<td>Handling resistance</td>
<td>–0.481</td>
<td>–0.209</td>
<td>–0.372</td>
<td>0.757</td>
<td>–0.105</td>
<td></td>
</tr>
<tr>
<td>Explained variance (%)</td>
<td>52.47</td>
<td>20.64</td>
<td>17.29</td>
<td>7.83</td>
<td>1.77</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Results of the model selection procedure for the association between boldness, coloration (plumage blackness and rump coloration) and size. Models are ranked according to AICc values. In the models including an interaction (*), single terms were also included.

<table>
<thead>
<tr>
<th>Variables</th>
<th>AICc</th>
<th>ΔAICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plumage blackness*Sex</td>
<td>452.68</td>
<td>0</td>
</tr>
<tr>
<td>Plumage blackness<em>Sex + Wing size</em>Sex</td>
<td>452.77</td>
<td>0.08</td>
</tr>
<tr>
<td>Plumage blackness*Sex + Body mass</td>
<td>454.70</td>
<td>2.02</td>
</tr>
<tr>
<td>Plumage blackness*Sex + Rump coloration</td>
<td>454.73</td>
<td>2.05</td>
</tr>
<tr>
<td>Plumage blackness*Sex + Wing size</td>
<td>454.83</td>
<td>2.14</td>
</tr>
<tr>
<td>Plumage blackness<em>Sex + Wing size</em>Sex + Rump coloration</td>
<td>454.84</td>
<td>2.16</td>
</tr>
<tr>
<td>Plumage blackness<em>Sex + Wing size</em>Sex + Body mass</td>
<td>454.92</td>
<td>2.24</td>
</tr>
<tr>
<td>Plumage blackness<em>Sex + Rump coloration</em>Sex + Wing size*Sex</td>
<td>455.63</td>
<td>2.95</td>
</tr>
<tr>
<td>Plumage blackness<em>Sex + Rump coloration</em>Sex</td>
<td>455.72</td>
<td>3.04</td>
</tr>
<tr>
<td>Plumage blackness<em>Sex + Body mass</em>Sex</td>
<td>456.10</td>
<td>3.42</td>
</tr>
</tbody>
</table>

Table 3. Results of the linear models exploring the association between boldnessc and plumage blackness or wing size in both male and female nestlings. Values for the excluded non-significant variables refer to the step before their exclusion.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Estimate</th>
<th>se</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females (n = 78)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plumage blackness</td>
<td>0.272</td>
<td>0.108</td>
<td>6.315</td>
<td>0.014</td>
</tr>
<tr>
<td>Wing size</td>
<td>0.019</td>
<td>0.015</td>
<td>1.671</td>
<td>0.200</td>
</tr>
<tr>
<td>Males (n = 70)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plumage blackness</td>
<td>–0.145</td>
<td>0.101</td>
<td>1.767</td>
<td>0.157</td>
</tr>
<tr>
<td>Wing size</td>
<td>–0.027</td>
<td>0.017</td>
<td>2.313</td>
<td>0.133</td>
</tr>
</tbody>
</table>

Figure 1. Relationship between boldnessc and plumage blackness in Common Kestrel nestlings. Boldnessc represents the mean-centred values of boldness to year and nest (see Methods for further details). The solid line and dots represent female nestlings, and the dashed line and triangles, male nestlings.

Juvenile plumage may also signal individual quality or condition to parents during the period of parental care (Senar 2006, Moreno & Soler 2011), as known for Kestrels (Vergara & Fargallo 2007). Any of these hypotheses may predict a correlation between coloration and boldness. However, it is still unclear whether juvenile plumages can act as a signal of status (Senar 2006, Fargallo et al. 2014), because status signals should work both within and between age and sex classes (Rohwer 1975). Our results show an association between plumage coloration and boldness in Common Kestrel fledglings, suggesting that juvenile plumage coloration could have evolved due to the pressures of status signalling.

We also found that this association was mediated by sex, as it was only positive and significant for female nestlings, providing evidence of a sex-dependent integration of personality and coloration. This differential
integration suggests that these traits are under different selection pressures in males and females. Plumage coloration works as an ornament in adult Common Kestrel males (Palokangas et al. 1994, López-Idiáquez et al. 2016b). The idea that a directional female preference for more ornamented males promotes directional selection in male ornaments might explain a greater detachment of plumage coloration from other traits in male Common Kestrels, resulting in a weak phenotypic integration (Pigliucci & Preston 2004). A sex–wing length interaction was also found in boldness behaviour, although the correlation was not significant for either of the sexes separately. In addition, we do not have data to distinguish the effect of growth or age of the chick on wing length. More precise data should be collected to investigate this potential sexual interaction.

Interestingly, our results show that rump coloration was not associated with our shy/bold behavioural axis. Rump coloration is an index of male competitive ability of offspring during the post-fledging dependence period (Vergara & Fargallo 2008). Fledglings with greyer rumps are able to capture a greater number of the prey provided by the parents than are fledglings with browner rumps (Vergara & Fargallo 2008). On the one hand, the lack of association may be explained by the fact that the ability to monopolize more prey does not necessarily have to be associated with personality and could reflect other characteristics, such as flight ability or condition. On the other hand, it is also possible that this lack of association may be explained by different personality axes that were not studied here, such as the proactive/reactive axis (David et al. 2011), rather than to the shy/bold axis.

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